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Antifungal activity of the essential oil of *Thymus capitellatus* against *Candida*, *Aspergillus* and dermatophyte strains

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ABSTRACT: The antifungal activity of *Thymus capitellatus* oils on *Candida*, *Aspergillus* and dermatophyte strains were studied. The essential oils were obtained from the aerial parts of the plants by water distillation and analysed by GC and GC–MS. Three chemotypes were characterized: 1,8-cineole (47.5%), 1,8-cineole/borneol (28.8% and 19.5%, respectively) and 1,8-cineole/linalyl acetate/linalool (27.5%, 20.0% and 17.0%, respectively). The minimal inhibitory concentration (MIC) determined according to the NCCLS protocols (M27-A and M38-P) and the minimal lethal concentration (MLC) were used to evaluate the antifungal activity of the oils against *Candida* (seven clinical isolates and three ATCC type strains), *Aspergillus* (five clinical isolates, two CECT and two ATCC type strains) and five dermatophyte clinical fungi strains. The oils exhibited antifungal activity for the dermatophyte strains, with MIC values of 0.32–1.25 μl/ml; the chemotype 1,8-cineole/linalyl acetate/linalool proved to be more active. The highest antifungal activity of this oil can be associated with the contribution of the linalyl acetate. In the other hand, all samples showed low activity against *Candida* and *Aspergillus* strains. Copyright © 2006 John Wiley & Sons, Ltd.

KEY WORDS: *Thymus capitellatus*; Lamiaceae; essential oil composition; antifungal activity

Introduction

Thymus capitellatus Hoffmanns. & Link (family Lamiaceae; local name 'tomilho do mato') is an endemic aromatic plant from Portugal[1] which grows in the estuaries and down-river parts of the Tejo and Sado basins (Estremadura, Ribatejo and Alentejo provinces).

Studies on the essential oils obtained from collective[2,3] and individual samples[4] of *T. capitellatus* demonstrated that this species is polymorphic, with three main chemotypes (1,8-cineole; 1,8-cineole/borneol and 1,8-cineole/linalyl acetate/linalool chemotypes). Our previous results showed that the 1,8-cineole and 1,8-cineole/borneol chemotypes are well distributed in the provinces of Ribatejo and Alentejo, whereas the 1,8-cineole/linalyl acetate/linalool chemotype is widespread only in the province of Estremadura. We verified that in Estremadura province other *Thymus* species, such as *T. carnosus* and *T. mastichina*, were also characterized by high linalool content, whereas other samples coming from various regions of Portugal have very low amounts of this compound.[5,6] This coast has a high Atlantic maritime humidity concentration, which seems to be correlated with the amount of linalool.

In some localities of the Estremadura, *T. capitellatus* is regarded as an antiseptic and is usually used in the treatment of cutaneous infections. The objective of the present research is to determine the antifungal activity of chemically well-defined chemotypes of *T. capitellatus* on *Candida*, *Aspergillus* and dermatophyte fungal strains. To our knowledge, this is the first report on the antifungal activity of the essential oil of this species.

Material and Methods

Plant Material

Aerial parts of the plants were collected at the flowering stage from two different localities of Ribatejo (Porto Alto, sample A, and Samora Correia, sample C) and one locality of Estremadura (Poceirão, sample B). Voucher

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specimens were deposited at the Herbarium of the Instituto Batânico of the University of Coimbra (COI), under Accession Nos LS 220–222.

Essential Oil Analysis

The essential oil content of the air-dried plant material was determined according to the *European Pharmacopoeia* method.[7] Analysis of volatile oils obtained by water distillation for 3 h were carried out by GC and GC–MS, using fused silica capillary columns with two different stationary phases: SPB-1 (polydimethylsiloxane 30 m × 0.20 mm i.d., film thickness 0.20 μm), and Supelco Wax 10 (polyethyleneglycol 30 m × 0.20 mm i.d., film thickness 0.20 μm); oven temperature programme, 70 °C to 220 °C at 3 °C/min, then held at 220 °C for 15 min; injector temperature, 250 °C; detector carrier gas, helium, adjusted to a linear velocity of 30 m/s; split ratio, 1:40; temperatures, 250 °C.

GC–MS was performed with a HP1 fused silica column (polydimethylsiloxane 30 m × 0.25 mm i.d., film thickness 0.25 μm), interfaced with a mass selective detector. GC parameters were as above; interface temperature, 250 °C; MS source temperature, 230 °C; MS quadrupole temperature, 150 °C; ionization energy, 70 eV; ionization current, 60 μA; scan range, 35–350 u; scans/s, 4.51.

The identity of the components was achieved from their retention indices, calculated by linear interpolation relative to retention times of a series of *n*-alkanes, and their mass spectra, which were compared with those from our own library and literature data.[8,9]

Relative amounts of individual components were calculated based on GC peak areas without FID response factor correction.

Antifungal Evaluation

Antifungal activity of the three chemotypes was evaluated against *Candida*, *Aspergillus* and dermatophyte strains: seven *Candida* clinical strains, two of *C. albicans* (M1, H37), one of *C. krusei* (H9), one of *C. tropicalis* (H18), one of *C. guillermondii* (Mat23) and two of *C. glabrata* (H16, H30) isolated from recurrent cases of vulvovaginal candidosis, as well as three ATCC type strains (*C. albicans* ATCC 10231, *C. tropicalis* ATCC 13803 and *C. parapsilosis* ATCC 90018); five *Aspergillus* clinical strains, one of *A. niger* (F01), three of *A. fumigatus* (F05, F07, F17) and one of *A. flavus* (F44) isolated from bronchial secretions, as well as two ATCC type strains (*A. niger* ATCC 16404 and *A. fumigatus* ATCC 46645) and two CECT type strains (*A. niger* CECT 2574 and *A. fumigatus* CECT 2071); five dermatophyte clinical strains (*Microsporum canis* FF1, *M. gypseum* FF3, *Trichophyton rubrum* FF5, *T. mentagrophytes* FF7 and *Epidermophyton floccosum* FF9) isolated from nails and skin. MICs and MLCs were determined by a macrodilution method according the NCCLS protocols (M27-A and M38-P).[10,11] Antifungal activity of the major constituents of each chemotype (1,8-cineole, borneol, linalyl acetate and linalool) were also evaluated against dermatophyte strains. Fluconazole (Sigma) and amphotericin B (Pfizer) were used to control the sensitivity of the tested microorganisms. The two-fold serial dilutions that were used varied in the range 128–0.25 μg/ml for fluconazole, 16–0.25 μg/ml for amphotericin B and 20–0.16 μl/ml (v/v) for the essential oils and their major components. Three independent experiments performed in duplicate were realized for each chemotype (A, B and C).

Results and Discussion

The oils were obtained in yields of 1.9–2.0% (v/w). The qualitative and quantitative compositions of the three chemotypes (A, B and C) used for antifungal evaluation are shown in Table 1, where the compounds are listed in order of their elution on a polydimethylsiloxane column. In total, 67 compounds were identified, accounting for 96.0–97.5% of the essential oils. The three chemotypes were characterized by a high percentage of oxygenated monoterpenes (67.5–81.9%) and monoterpene hydrocarbons (10.5–22.2%). Sesquiterpenes attained 5.1–7.2%. All three oils had a high 1,8-cineole content (27.5–47.5%). Nevertheless, there were some important differences between them. In chemotype A the 1,8-cineole was the major component (47.5%), whereas in the chemotypes B and C there were other main compounds: in chemotype B linalyl acetate (20.0%) and linalool (17.0%) and in chemotype C borneol.

Evaluation of minimal inhibitory concentration (MIC) and minimal lethal concentration (MLC) of the three chemotypes of *T. capitellatus* showed antifungal activity against dermatophyte strains, with MIC and MLC values of 0.32–1.25 μl/ml (Table 2). Nevertheless, the 1,8-cineole/linalyl acetate/linalool chemotype (B) proved to be more active. Antifungal activity of the major constituents of the three chemotypes (1,8-cineole, borneol, linalyl acetate and linalool) were also assayed against the same dermatophytes. The highest antifungal activity of chemotype B can be associated with the contribution of the linalyl acetate (Table 3). These results support the popularity of this plant in the treatment of cutaneous infections and may justify the future use of *T. capitellatus* oils, particularly these with high amounts of linalyl acetate, in the treatment of dermatophytosis. For *Candida* and *Aspergillus* strains the three oils showed low activity, with MIC values of 1.25–10.0 μl/ml (Table 2). *C. tropicalis* and *A. flavus* showed higher resistance to the oils.

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Table 1. Percentage composition of three chemotypes of *T. capitellatus* from Portugal

Compound	RI[a]	RI[b]	Content (%)		
			A	B	C
Tricyclene	922	1030	0.3	0.1	0.2
α-Thujene	923	1030	0.5	0.1	0.5
α-Pinene	930	1030	4.9	1.7	5.0
Camphene	943	1077	3.8	0.4	5.2
Sabinene	969	1127	2.3	1.8	3.8
β-Pinene	970	1118	3.9	1.7	2.9
Myrcene	981	1164	1.1	1.2	0.7
α-Phellandrene	998	1171	0.1	t	t
α-Terpinene	1009	1187	0.3	0.3	0.3
p-Cymene	1012	1275	0.5	0.3	0.4
Limonene	1020	1207	1.0	0.6	0.9
1,8-Cineole	1020	1215	47.5	27.5	28.8
(*Z*)-β-Ocimene	1025	1235	0.2	0.5	0.2
(*E*)-β-Ocimene	1035	1253	1.4	0.7	0.9
γ-Terpinene	1047	1248	1.0	0.7	0.9
trans-Sabinene hydrate	1050	1459	0.5	t	0.6
cis-Linalool oxide	1056	1442	0.1	0.9	0.1
Fenchone	1066	1400	t	t	0.1
trans-Linalool oxyde	1070	1466	t	0.5	0.1
cis-Sabinene hydrate	1077	1464	0.2	0.3	0.3
Terpinolene	1077	1288	0.4	0.3	0.2
Linalool	1084	1544	2.5	17.0	3.0
α-Campholenal	1102	1487	0.1	t	0.1
Camphor	1118	1515	2.0	0.6	4.0
trans-Pinocarveol	1119	1647	0.9	0.4	0.5
trans-Verbenol	1126	1664	0.7	0.4	0.7
Pinocarvone	1135	1553	0.3	t	0.2
δ-Terpineol	1144	1667	0.8	0.8	1.1
Borneol	1146	1696	3.4	0.6	19.5
p-Cimene-8-ol	1158	1845	t	0.1	0.1
Terpinene-4-ol	1159	1599	1.7	1.0	2.4
Myrtenal	1165	1622	0.4	t	0.3
α-Terpineol	1169	1693	1.8	3.5	3.3
Verbenone	1176	1696	0.1	0.3	0.1
Myrtenol	1176	1786	0.3	t	0.2
trans-Carveol	1195	1830	t	0.1	0.2
Nerol	1209	1797	0.1	1.5	0.1
Carvone	1211	1728	t	t	0.2
Neral	1214	1679	—	0.1	t
Geraniol	1233	1842	t	1.5	0.1
Linalyl acetate	1240	1555	t	20.0	t
Bornyl acetate	1266	1578	1.5	0.4	1.5
α-Terpenyl acetate	1328	1688	2.3	2.0	0.9
Neryl acetate	1342	1722	t	0.7	t
α-Cubebene	1342	1457	t	t	t
Geranyl acetate	1359	1755	t	1.5	t
β-Bourbonene	1376	1517	t	0.1	0.1
β-Cubebene	1381	1536	t	—	t
β-Elemene	1382	1585	0.2	0.1	0.1
α-Gurjunene	1402	1527	0.1	0.1	0.1
(*E*)-Caryophyllene	1410	1594	0.4	0.1	0.3
α-Humulene	1442	1664	0.1	—	0.2
allo-Aromadendrene	1449	1639	0.3	0.1	0.2
Germacrene D	1466	1699	0.1	0.4	0.1
Bicyclogermacrene	1482	1726	0.1	t	t
β-Bisabolene	1495	1723	0.2	t	0.1
γ-Cadinene	1498	1751	0.1	0.1	0.1
δ-Cadinene	1508	1751	0.2	t	t
Elemol	1526	2070	0.4	0.2	0.3
Caryophyllene oxide	1558	1968	0.5	0.2	0.6
Viridiflorol	1572	2073	1.3	1.0	0.4
Ledol	1582	2024	0.4	0.9	0.2
10-*epi*-γ-Eudesmol	1594	2089	0.1	0.2	0.1
Cubenol	1605	2052	0.2	0.2	0.1
T-Cadinol	1615	2160	0.1	0.1	0.1
α-Cadinol	1628	2220	0.1	0.2	0.1
Intermedeol	1630	n.d.	2.2	1.0	1.8

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Table 1. (*Continued*)

Compound	RI[a]	RI[b]	Content (%)		
			A	B	C
Monoterpene hydrocarbons			21.7	10.5	22.2
Oxygen-containing monoterpenes			67.5	81.9	68.6
Sesquiterpene hydrocarbons			1.9	1.1	1.5
Oxygen-containing sesquiterpenes			5.3	4.0	3.7
Total identified			96.4	97.5	96.0

Compounds listed in order to their elution on the SPB-1 column.
t, trace (<0.05%).
RI[a], Retention indices on the SPB-1 column relative to C_8–C_{22} *n*-alkanes.
RI[b], Retention indices on the SupelcoWax-10 column relative to C_8–C_{22} *n*-alkanes.
n.d., not determined.

Table 2. Antifungal activity (MIC and MLC) of the three chemotypes of *T. capitellatus* for *Candida*, dermatophyte and *Aspergillus* strains

Strains	Chemotype A		Chemotype B		Chemotype C		Fluconazole		Amphotericin B	
	MIC[a]	MLC[a]	MIC	MLC	MIC	MLC	MIC[b]	MLC[b]	MIC	MLC
Candida albicans ATCC 10231	2.5–5.0	2.5–5.0	2.5	2.5–5.0	2.5–5.0	2.5–5.0	1	>128	N.T[c]	N.T
C. albicans H37	1.25–2.5	2.5	1.25	1.25	1.25–2.5	1.25–2.5	64	>128	N.T	N.T
C. albicans M1	2.5–5.0	2.5–5.0	2.5	5.0	2.5–5.0	2.5–5.0	2	128	N.T	N.T
C. tropicalis ATCC 13803	5.0	5.0	2.5–5.0	5.0	2.5–5.0	5.0	4	>128	N.T	N.T
C. tropicalis H18	5.0	5.0	2.5–5.0	2.5–5.0	2.5–5.0	2.5–5.0	2	>128	N.T	N.T
C. glabrata H16	2.5	2.5–5.0	1.25–2.5	2.5	1.25–2.5	1.2–2.5	16	16	N.T	N.T
C. glabrata H30	5.0	5.0	2.5	5.0	2.5–5.0	5.0	32	32	N.T	N.T
C. krusei H9	2.5–5.0	5.0	2.5–5.0	2.5–5.0	1.25–2.5	2.5	64	64–128	N.T	N.T
C. guillermondii MAT23	2.5	2.5	2.5	2.5	2.5	2.5	8	8	N.T	N.T
C. parapsilosis ATCC 90018	2.5–5.0	5.0	2.5	2.5–5.0	5.0	5.0	<1	<1	N.T	N.T
Epidermophyton floccosum	0.64	0.64	0.32	0.32	0.64	0.64	16	16	N.T	N.T
Trichophyton rubrum	0.64	1.25	0.64	1.25	1.25	1.25	16–32	32	N.T	N.T
T. mentagrophytes	0.64	0.64	0.64	0.64	0.64	0.64–1.25	16–32	32–64	N.T	N.T
Microsporum canis	0.64	0.64	0.64	0.64–1.25	1.25	1.25	128	128	N.T	N.T
M. gypseum	1.25	1.25	0.64	0.64–1.25	0.64	1.25	≥128	≥128	N.T	N.T
Aspergillus niger ATCC 16404	5.0	5.0–10.0	2.5	20.0	5.0	20.0	N.T	N.T	1–2	4
A. niger CECT 2574	5.0	5.0–10.0	2.5	20	5.0	20.0	N.T	N.T	2	4
A. niger F01	5.0	10.0	2.5	10.0	5.0	10.0	N.T	N.T	1	2
A. fumigatus ATCC 46645	2.5–5.0	10.0	2.5	10.0	2.5	10.0	N.T	N.T	2	4
A. fumigatus CECT 2071	2.5–5.0	5.0–10.0	2.5	10.0	2.5–5	10.0	N.T	N.T	1–2	4
A. fumigatus F05	5.0	10.0	2.5	10.0	2.5–5	10.0	N.T	N.T	2–4	4–8
A. fumigatus F07	5.0	20.0	2.5	10.0	2.5–5	10.0	N.T	N.T	2–4	4
A. fumigatus F17	5.0	10.0	2.5	10.0	2.5	10.0	N.T	N.T	2	4–8
A. flavus F44	10.0	≥20	5.0	≥20.0	10.0	10–20.0	N.T	N.T	2	8

[a] MIC and MLC were determined by a macrodilution method and expressed in µl/ml (v/v).
[b] MIC and MLC were determined by a macrodilution method and expressed in µg/ml (w/v).
[c] Not tested.

Table 3. Antifungal activity (MIC and MLC) of the major compounds of the essential oil of *T. capitellatus* for dermatophyte strains

Strains	1,8-Cineole		Borneol		Linalool		Linalyl acetate	
	MIC[(a)]	MLC[(a)]	MIC	MLC	MIC	MLC	MIC	MLC
Epidermophyton floccosum	5.0	5.0	2.5	2.5–5.0	1.25–2.5	2.5	0.32	0.32
Trichophyton rubrum	2.5–5.0	2.5–5.0	2.5	2.5–5.0	1.25	1.25	0.32–0.64	0.32–0.64
T. mentagrophytes	5.0	5.0	2.5	2.5–5.0	1.25	2.5	0.64	0.64
Microsporum canis	5.0	5.0	2.5	2.5–5.0	2.5	2.5	0.32–0.64	0.32–0.64
M. gypseum	10.0	10.0	2.5	2.5–5.0	2.5	2.5	0.64	0.64

[a] MIC and MLC were determined by a macrodilution method and expressed in µl/ml (v/v).
[b] MIC and MLC were determined by a macrodilution method and expressed in µg/ml (w/v).

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